reserves the right to file a divisional application for the non-elected invention set forth in the canceled claims.

Applicant notes the Examiner's acknowledgment of Applicant's claim for foreign priority under 35 U.S.C. § 119 and receipt of the certified priority document. Claims 1-12, 19-27 and 31-33 are pending in this application.

Rejection Under 35 U.S.C. 103

Claims 1-12, 19-27 and 31-33 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Orlefors et al. (WO 00/40750) in view of the Maeshima publication. Applicant respectfully traverses the rejection. According to the Examiner, Orlefors et al. teach a method comprising the steps of preparing a sample solution with a primer that binds to a nucleic acid and at least a ddNTP or dNTP, allowing the solution to sit to cause an extension reaction, and measuring the progress of the reaction by measuring the PPi produced.

Orlefors et al. disclose a CD device for discriminating a single nucleotide polymorphism (SNP) or sequencing a DNA by running a DNA extension reaction and detecting the release of PPi. The reference discloses several methods for detecting PPi. The preferred method is to use a luciferase reaction (see pages 9-10 of Orlefors et al.).

In the background of the invention, Applicant acknowledges the luciferase reaction is known, but points out the problem when dATP is used in the primer extension reaction. In such a reaction, dATP becomes a substrate for a luciferase reaction, and a special dATP analogue must be used which acts as substrate for the DNA polymerase instead of dATP and does not act as substrate for the luciferase reaction (see page 12, lines 16-26 of Orelfors et al). Orlefors et al. do not disclose use of H+ pyrophosphatase (hereinafter H+-PPase). The Examiner concedes this

fact. The Examiner has held that Orelfors et al. do not disclose "contacting [the] sample solution with a permeable membrane having H^+ pyrophosphatase which hydrolyses pyrophosphate released during extension reaction and measuring the H^+ concentration either in solution on the front face of the membrane or in the solution at the back face of the membrane." For this deficiency, the Examiner relies on the teachings of Maeshima.

Maeshima does not make up for the deficiency of Orlefors et al. Maeshima describes H+-PPase in general. It describes that the reaction of H+-PPase with PPi will transport H+, but the reference neither discloses nor suggests the use of such a reaction for detection of DNA extension reaction. More importantly, the reference does not disclose the possible reaction of H+-PPase with dATP, dTTP, dCTP and dGTP, which are substrates for DNA extension reaction and which contain PPi within their molecular structures and thus are highly likely to react with H+-PPase. Therefore, it would not have been obvious to a person skilled in the art to substitute H+-PPase disclosed in Maeshima in place of the luciferase system disclosed in Orlefors et al.

The Examiner asserts that Maeshima at page 39, col. 1, section 2-2 teaches "measuring the activity of H^+-PPase based on PPi hydrolysis." The passage relied upon by the Examiner states, in pertinent part, that

> The actual free energy change for PPi hydrolysis in the cytoplasm has been calculated to be 27.3 kJ/mol at pH 7.3 [21]. The H^+/PPi stoichiometry of H^+-PPase has been determined to be 1, and the steady-state pH generated by the enzyme 3.2 [22]. Specific activities of H^+-PPase in vacuolar membranes vary with the plant species, tissues, and assay conditions. Typical values are 1.10, 0.30, 0.52, 0.35, 1.56, and 0.22-0.71 μmol PPi/min per mg of membrane protein for the seedling hypocotyl of mung bean [15], storage tissue of red beet [16,17], *Arabidopsis* leaf [23], cotyledon of pumpkin seedling [24], *Acetabularia* [25], and CAM plants [19], respectively. The actual substrate for H^+-PPase is a Mg^{2+}-PPi complex (Mg2PPi) [1,26-28]. The purified enzyme requires phospholipid for catalysis [15-17]. The specific activities of PPi hydrolysis by the purified H^+-PPases of mung bean and red beet were 8.5 and 3.0 (jmol/min per mg, respectively. The enzyme purified from CAM plants also has shown similar values [19]. A high specific

> activity of 20 (μmol/min per mg has been reported for the purified preparation of *Rhodospiril-lum rubrum* H^+-PPase [6]. The half-maximum activity of the purified enzyme was obtained at 35μM MgPPi in the presence of 1 μM Mg^{2+} in the assay medium. H^+-PPase in the purified form and in the vacuolar membrane expresses its maximal velocity at more than 200 μM PPi in the presence of 1 μM $MgSO_4$. From kinetic analysis, the measured K_m for the substrate has been reported to be 130 μM [29], 2-5 μM [27], and 2 μM [26]. The reported value of the cytosolic PPi concentration of approximately 200 μM is enough for expression of maximal activity of H^+-PPase in plant cells.

The Examiner has not explained how this passage would have led a person having ordinary skill in the art to use a membrane having H+-PPase to measure the activity of H+-PPase based on PPi hydrolysis as required by base claims 1 and 19.

The Examiner further finds at page 45, col. 1, paragraph 1, Fig. 4, that "Maeshima also teaches that the active synthesis in growing tissue results in large amount of PPi production as a by product, which is scavenged by vacuolar H^+-PPase and is used as a source of energy for active transport of protons into the expanding vacuoles." The relevance of this teaching to the claimed subject matter has not been explained in the Office Action.

Examiner concludes from the teachings of Maeshima that "[i]t would have been prima facie obvious to a person of ordinary skill in the art at the time the invention was made to modify the method of detecting extension product or base sequence based on PPi release as disclosed by Orlefors et al. with the step of including membrane associated H^+ pyrophosphatase to measure PPi release as disclosed by Maeshima for the purpose of developing a sensitive method for stably detecting base extension reaction or polymerization reaction of a target nucleic acid." The Office Action does not provide any cogent reasoning to explain how this conclusion is supported by the teachings of Maeshima. Moreover, the specification of the present application does not state that an object of the present invention is to develop a "sensitive" method. The reference fails to

disclose or suggest bringing the sample solution into contact with the membrane having H+-PPase to measure the H^+ concentration on either side of the membrane as required by base claims 1 and 19. The suggestion could only have come from Applicant's specification.

The Office Action further concludes that "[a]n ordinary person skill in the art would have been motivated to combine the method taught by the Orlefors et al. with the inclusion of vacuolar membrane associated H^+ pyrophosphatase to measure the release of PPi to achieve expected advantage of developing a sensitive method for detecting base sequence of a target nucleic acid." Again, the Office Action fails to provide cogent reasoning to set forth the factors that would have motivated the person skilled in the art without relying on Applicant's disclosure for the suggestion to use a vacular membrane. There is no teaching in Maeshima that the membrane can be used to measure PPi. While Maeshima discloses that the "substrate for H+-PPase, PPi, [sic] is produced as a by-product of several processes, such as polymerization of DNA and RNA ..." (p. 38, col. 1), the reference does not suggest or teach that the inclusion of vacuolar membrane associated H+-PPase can be used to measure the release of PPi in a DNA extension reaction.

The Office Action still further concludes that the "ordinary artisan would have a reasonable expectation of success that the inclusion of said vacuolar membrane associated H^+ pyrophosphatase would result in a sensitive measurement of PPi released during extension reaction by measuring H^+ concentration on at least either one side of the membrane because Maeshima explicitly taught that the by-product produced by polymerization of DNA or RNA is scavenged by vacuolar H^+-PPase, and uses it as a source of energy for transporting protons into the vacuoles (see page 38, col. 1, line 21-27, page 45, col. 1, paragraph 1, Fig. 4) and such modification of the method would be obvious over the cited prior art in the absence of secondary considerations." The

Examiner has not provided any cogent reasoning to explain how a person having ordinary skill in the art would have been led to conclude from the teaching of the reference that PPi is "scavenged" by H+-PPase. The passage relied upon by the Examiner merely states that PPi is the substrate for H^+-PPase. It does not disclose or suggest that H^+-PPase can be used as measure of the PPi produced from the polymerization of DNA and RNA. That suggestion could only have come from Applicant's disclosure.

The Office Action at page 4, line 4 states that "Orlefors et al teach that the PPi release is measured as a primer extension product produced, which is detected electrically." However, such a teaching cannot be found at cited pages referred to in the Office Action.

For all of the foregoing reasons, the combined teachings of Orlefors et al. and Maeshima do not present a *prima facie* case of obviousness. Accordingly, it is respectfully requested that the rejection of claims 1-12, 19-27 and 31-33 under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

Double Patenting Rejection

Claims 1-12, 19-27 and 31-33 stand provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1, 3-9, 14-19 of copending patent application No. 10/727,664 (U.S. Publication No. 2004/0197803) (hereinafter the '803 application). The Examiner did not approve the previously submitted Terminal Disclaimer because of an error in the Application No. referred to in the body of the document. A corrected Terminal Disclaimer is submitted with this response. It is respectfully requested that the Terminal Disclaimer submitted with this response be approved and entered, and that the provisional obviousness-type double patenting rejection be withdrawn.

Conclusion

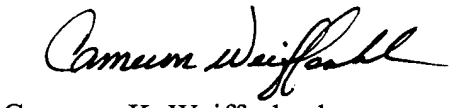
It is submitted that the claims 1-12, 19-27 and 31-33 are patentable over the teachings of the prior art relied upon by the Examiner. Accordingly, favorable reconsideration of the claims is requested in light of the preceding remarks. Allowance of the claims is courteously solicited.

If there are any outstanding issues that might be resolved by an interview or an Examiner's amendment, the Examiner is requested to call Applicants' attorney at the telephone number shown below.

To the extent necessary, a petition for an extension of time under 37 C.F.R. § 1.136 is hereby made. Please charge any shortage in fees due under 37 C.F.R. § 1.17 and due in connection with the filing of this paper, including extension of time fees, to Deposit Account 500417 and please credit any excess fees to such deposit account.

Respectfully submitted,

McDERMOTT WILL & EMERY LLP



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